Food Research International 63 (2014) 55–61

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Modification of rice grain starch for lump-free cooked rice using thermostable disproportionating enzymes

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ARTICLE INFO

Article history:
Received 5 June 2013
Received in revised form 29 March 2014
Accepted 13 April 2014
Available online 24 April 2014

Keywords:
lump-free cooked rice and rice porridge
thermostable enzyme
disproportionating enzyme
rice starch
alpha-glucanotransferase
cyclodextrin glucanotransferase

ABSTRACT

Thermostable disproportionation enzymes active at around the gelatinization temperature of rice starch (70–80 °C) were used to improve the quality of cooked rice with lump-free properties. Cyclodextrin glucanotransferase from *Pyrococcus furiosus* (CGTase) and a starch binding domain-attached alpha-glucanotransferase from *Thermus aquaticus* (αGTase) were prepared from an *Escherichia coli* transformant carrying a recombinant plasmid. The stickiness determined by a texture analyzer showed that both enzymes had the effect of reducing the lumping properties of cooked rice grains and rice porridge. The enzyme treatment also provided a finer, creamier, and more uniform texture of rice porridge. An HPAEC analysis of the starch structure in cooked rice and rice porridge revealed that the proportion of both short (DP < 6) and long-branch chains (DP > 20) increased in the enzyme-treated samples. Additionally, the amylose content of the cooked rice grain treated with αGTase and CGTase decreased by 36% and 30%, whereas that of porridge was reduced by 36% and 35%, respectively. In particular, the amylose content on the surface of the enzyme-treated cooked rice grains was reduced by 50% compared with that of untreated cooked rice. These results indicated that the glucan segment of amylose was transferred to amylopectin branch chains by a transferring reaction with both enzymes, reducing the amylose content of grains during cooking. Mechanistically, the decrease in the amylose content filmed on the surface is likely to be responsible for reducing the stickiness of cooked rice grains and rice porridge. Furthermore, the retrogradation rate determined by a differential scanning calorimeter was significantly reduced in the enzyme-treated samples after storage for 10 h at 4 °C. The decrease in long-chain amyloses, may lead to the retardation of re-crystallization. These physicochemical properties of the modified cooked rice and rice porridge can be applied to various rice-based products in the food industry.

1. Introduction

In many countries, rice is conventionally consumed in a boiled or steamed form. A better understanding of the texture and appearance of cooked rice grains is of great importance to consumer acceptability, as both vary depending on the method of cooking (Bharath Kumar & Prabhasankar, 2014). The cooking of rice involves water diffusion into rice grains during starch gelatinization, resulting in structural changes (Riva, Schirald, & Piazza, 1994). Furthermore, several studies have shown that the change in amylose content in rice grains has a significant impact on the texture and taste of cooked rice. During the cooking of rice, amylose is leached outward and creates a film on the surface of rice grains, contributing to the stickiness of cooked rice (Leelayuthsoontorn & Thipayarat, 2006). We observed that the increase in the stickiness of cooked rice was a function of the cooking temperature (Cho, Auh, Kim, et al., 2009; Cho, Auh, Ryu, et al., 2009). In many Asian countries, cooked rice is frequently served in a bowl topped with seasoned vegetables, sliced meat, and sauces. Often these ingredients are thoroughly mixed together with cooked rice just before eating. However, when placed on the dining table for a short period, cooked rice grains rapidly clump together. Techniques for preparing lump-free rice have received

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http://dx.doi.org/10.1016/j.foodres.2014.04.007
0963-9969/

considerable attention, especially in Northeast Asia, because the staple rice variety in this area is short-grain rice, which has sticky characteristics. Traditionally, one of the "de-lumping" processes developed in culinary technology for porridge is continuous stirring at low heat. However, there are few published studies of the fine structure of rice starch in relation to the non-lumping properties of cooked rice. Our previous studies have shown that the structural properties of isolated cereal starches were changed by enzymes such as αGTase and amylolytic enzyme. (Cho, Auh, Kim, et al., 2009; Cho, Auh, Ryu, et al., 2009; Kim, Mun, Park, Shim, & Kim, 2013; Park, Park, & Jane, 2007; Seo, Roh, Park, Kim, & Park, 2007; Shim et al., 2007; Van der Maarel & Leemhuis, 2013). However, the enzymatic modification of cooked rice grains may differ from that of isolated starch.

To improve cooked rice, a mixture of various enzymes such as transglutaminase and/or α-glucosidase has been used together with glucose oxidase to remove the by-product, glucose (Yamada, Sato, & Kodera, 2011). The mixture of three different enzymes may require complicated processing conditions with different optimum temperatures and pH levels for each enzyme. Because the enzyme treatments typically result in a high glucose level, their dried product may be off-color due to browning reactions between the cereal proteins and glucose. Similarly, a significant amount of glucose and maltose was produced when maltogenic amylase was added to reduce the amylose content in rice starch (Auh et al., 2006). Furthermore, enzyme treatments tend to yield a "watery" porridge (Gantwerker & Leong, 1984). Unlike hydrolyzing enzymes, disproportionating enzymes transfer the glucan molecule to other acceptor molecules, thereby minimizing glucose formation in the end products. Therefore, disproportionating enzymes may be very effective in reducing amylose in rice starch without producing glucose as a by-product. It is known that the enzymes, α-GTase (EC 2.4.1.25), and CGTase (EC 2.4.1.19) catalyze the transfer of a fragment of glucan to the hydroxyl group of the acceptor via the formation of α-1,4-glucosidic linkage:

$$(\alpha\text{-}1,4\text{-glucan})_a + (\alpha\text{-}1,4\text{-glucan})_b \rightarrow (\alpha\text{-}1,4\text{-glucan})_{a\text{-}x} + (\alpha\text{-}1,4\text{-glucan})_{b+x}.$$

When D-glucose is present, it can be elongated to longer oligosaccharides by the transfer of a glucan moiety from donor molecules:

$$(\alpha\text{-}1,4\text{-glucan})_a + \text{D-glucose} \rightarrow (\alpha\text{-}1,4\text{-glucan})_{a\text{-}x} + (\alpha\text{-}1,4\text{-glucan})_{1+x}.$$

Furthermore, when using enzymes in cooking rice, the thermostable enzyme is preferred because the enzyme reaction needs to be maintained above the gelatinization temperature of rice starch (Gantwerker & Leong, 1984). We have previously investigated the structural modification of starch by thermostable enzymes including thermostable CGTase, maltogenic amylase (MAase), and α-GTase (Park, kim, et al., 2007; Shim et al., 2007).

Previously we cloned a thermostable αGTase from *Thermus aquaticus* (optimum temperature = 70 °C) and introduced the starch-binding domain to enhance starch utilization activity (Park, Kim, et al., 2007). A highly thermostable CGTase with an optimal temperature of 95 °C was also cloned from *Pyrococcus furiosus* and successfully overexpressed (Lee et al., 2007).

Recently, isolated rice starch has been modified by αGTase (Cho, Auh, Kim, et al., 2009; Cho, Auh, Ryu, et al., 2009). However, the study of isolated rice starch provides only limited information for the evaluation of the quality of cooked rice because rice grains are composed of various components such as starch, proteins, and lipids that affect its texture when cooked. Therefore, in this study we investigated the effect of thermostable disproportionating enzymes on various physicochemical properties of cooked rice grains and their use to produce lump-free cooked rice.

2. Materials and methods

2.1. Materials

Rice used in this study was purchased from the market in Seoul, Korea. Isoamylase from *Pseudomonas amyloderamosa* was purchased from Sigma-Aldrich (St. Louis, MO, USA), and all other chemicals were of analytic grade.

2.2. Preparation of αGTase

The *Escherichia coli* (MC1061) transformant carrying a recombinant plasmid (p × 6 His-TAαGT-DE) (Liebl, Feil, Gabelsberger, Kellermann, & Schleifer, 1992; Park, Kim, et al., 2007) was cultivated in LB medium containing ampicillin (100 μg/ml). The cells were harvested by centrifugation and resuspended in lysis buffer [50 mM Tris–HCl buffer (pH 7.5) containing 300 mM NaCl and 10 mM imidazole]. The cell extract was obtained by sonication (VC-600, Sonics & Materials, Newtown, CT, USA) and heated at 65 °C for 20 min. After centrifugation, the supernatant was passed through a nickel–nitrilotriacetic acid (Ni-NTA) column (Qiagen, Hilden, Germany). The column was washed with 50 mM Tris–HCl buffer (pH 7.5) containing 300 mM NaCl and 20 mM imidazole, followed by elution with 50 mM Tris–HCl buffer (pH 7.5) containing 300 mM NaCl and 250 mM imidazole. The purified protein was dialyzed against 50 mM Tris–HCl buffer (pH 7.5).

2.3. Preparation of CGTase

CGTase was purified from pTKPFCGT-carrying *E. coli* using a four-step procedure that included heat treatment. *E. coli* harboring pTKPFCGT (Lee et al., 2007) were grown in LB broth supplemented with kanamycine (100 μg/ml) and chloramphenicol (30 μg/ml) at 37 °C for 19 h with agitation (200 rpm). The cells were harvested via centrifugation and then sonicated (VC-600, Sonics & Materials, Newtown, CT, USA). The extract was incubated at 60 °C for 20 min and then passed through a Q-sapharose column (10 × 1.0 cm), a DEAE-Toyopearl column, and a gel permeation chromatography (GPC) column (30 × 1.0 cm) using an AKTA FPLC purifier system (Amersham Pharmacia, Uppsala, Sweden). The anion exchange columns were equilibrated with 50 mM Tris–HCl (pH 7.5 or 9.0), and the proteins were eluted with a linear gradient of NaCl (0–1 M).

2.4. Assay for αGTase activity

The activity of αGTase was determined by measuring the optical change in iodine staining during the conversion of amylose by the enzyme (Park, Kim, et al., 2007; Park, Kim, et al., 2007). The enzyme reaction mixture containing 250 μl of 0.2% (w/v) amylose, 50 μl of 1% (w/v) maltose, 600 μl of 50 mM Tris–HCl buffer (pH 7.5), and 100 μl of enzyme solution was incubated at 70 °C for 10 min. The reaction was stopped by boiling for 10 min. Aliquots (0.1 ml) were mixed with 1 ml of iodine solution [0.02% (w/v) iodine and 0.2% (w/v) potassium iodide], and the absorbance at 620 nm was measured immediately with an UV/visible spectrophotometer UV-160A (Shimadzu Corporation, Japan). One unit of αGTase activity was defined as the amount of enzyme that degraded 0.5 mg/ml of amylose per min under the assay conditions used. The protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin as a standard (Bradford, 1976).

2.5. Assay for CGTase activity.

CGTase activity was assayed using the phenolphthalein test described by Kaneko, Kato, Nakamura, and Horikoshi (1987). Enzyme solution (20 μL) was added to 1 mL of gelatinized soluble starch solution [4% (w/v) in 50 mM sodium acetate buffer (pH 5.0)] and incubated at 90 °C for 10 min. The reaction was stopped by adding 3.5 mL of